PERGAMON

Vision Research 38 (1998) 2881–2888

Vision Research

Water soluble antioxidants in mammalian aqueous humor: interaction with UV B and hydrogen peroxide

Stuart P. Richer [a,b,*], R.C. Rose [b]

[a] *Department of Veteran Affairs Medical Center, Eye Clinic 112E, North Chicago, IL 60064-3095, USA*

[b] *Department of Physiology and Biophysics, FUHS/Chicago Medical School, North Chicago, IL 60064-3095, USA*

Received 29 April 1997; received in revised form 27 August 1997; accepted 19 January 1998

Abstract

HPLC/electrochemical detection was used to identify five major low MW water soluble electrochemically active molecules from the aqueous humor of three species of mammals: New Zealand White rabbits and humans (diurnal) and Sprague–Dawley rats (nocturnal). These molecules are L-cysteine (CYS), L-ascorbic acid (AA), glutathione (GSH), uric acid (UA) and L-tyrosine (TYR); all of these molecules have known antioxidant properties. Nocturnal rat aqueous humor is concentrated in two thiols: GSH (125 μM; $n = 24$ pooled eyes) and CYS (63 μM), in contradistinction to diurnal species which have high concentrations of AA. No deterioration of any of these antioxidants occurs in a synthetic aqueous humor mixture irradiated with a physiologically relevant spectral UV B dose of 30 mJ/cm^2/h (5.5 UV equivalent sunlight hours). The same result occurred with addition of the endogenous aqueous humor UV B photosensitizer L-tryptophan. In a second set of experiments, human synthetic aqueous humor was subjected to hydrogen peroxide induced oxidant stress. The decay of antioxidants was CYS > GSH > AA > UA > TYR. The second highest concentrated antioxidant in human aqueous humor is TYR. Yet TYR failed to protect AA against H_2O_2-induced free radical damage in a synthetic aqueous humor model system ($P = 0.10$; ANOVA). The existence of multiple electrochemically active constituents and their thermodynamic interactions must be recognized when choosing animal models to evaluate human aqueous humor antioxidant defense.

Keywords: Antioxidants; Aqueous humor; Hydrogen peroxide; Ultraviolet B radiation

1. Introduction

To understand the mechanism of defense against free radical and oxidant damage, our laboratory has been involved in the identification and characterization of major water soluble antioxidants in liver, kidney, pancreas and intestinal mucosa with emphasis on the transport and metabolism of ascorbic acid [1–4]. In ocular tissue, we have turned our attention to the major low MW water soluble electrochemically active components in vitreous humor from New Zealand (NZ) rabbits, cows and Sprague–Dawley (SD) rats, [5] and in human tears [6]. One purpose of this paper is to compare mammalian inter-species composition of aqueous humor low MW antioxidants. Secondly, a synthetic aqueous humor solution containing these components was subjected to two forms of physiologically relevant oxidant stress: UV B radiation and hydrogen peroxide.

Aqueous humor is protected against free radical and oxidant stress by a system that includes antioxidant enzymes, chelating proteins and antioxidants, both lipid and water soluble. Identification and quantification of low MW water soluble protective factors is important in understanding how aging, disease and nutritional effects alter the concentrations of one or more components such as ascorbic acid [7–9]. The mechanisms of hydrogen peroxide detoxification in aqueous humor and lens, including the role of ascorbic acid and glutathione, have garnered attention [10,11]. The so called 'pecking order' for aqueous humor antioxidants, is influenced by the individual concentrations and one electron reduction potentials [12]. Inter-species differences in concentrations of aqueous humor electrochemically active species is of paramount importance in choosing appropriate animal models to study antioxi-

* Corresponding author. Tel.: +1 847 6881900, ext. 5406; fax: +1 847 5786924; e-mail: Richer,s@n-chicago.va.gov.

0042-6989/98/$19.00
PII: S0042-6989(98)00069-8

dant protective systems. Such models might help us understand ocular disorders such as uveitis, cataract and glaucoma.

We confirm the existence of five electrochemically active molecules in aqueous humor: ascorbic acid, glutathione, the amino acids L-cysteine and L-tyrosine and the purine metabolite uric acid. These occur in three species of mammals: NZ White rabbits and human cataract patients (diurnal) and SD rats (nocturnal). Halliwell describes L-tyrosine as a singlet oxygen quencher and notes that L-tyrosine and aromatics in general are potent hydroxyl radical scavengers [13]. This is significant, because in human aqueous humor, L-tyrosine is second highest in concentration next to ascorbic acid.

2. Materials and methods

2.1. Reagents

Phosphoric acids were obtained from Fisher; L-cysteine, L-ascorbic acid, glutathione, uric acid, L-tyrosine, M-tyrosine, DNTB (5,5[1] dithio-bis (2-nitrobenzoic acid), uricase, mushroom tyrosinase (EC # 1.14.18.1), desferrioxamine B, L-trytophan and hydrogen peroxide were obtained from Sigma (St. Louis, MO).

2.2. Chromatographic system

Aliquots of 20 μl were injected onto an HPLC electrochemical detection system that has been described in detail [14]. Briefly, the chromatograph consists of a Beckman Model 110B solvent pump, Waters 710B injector, Waters Resolve C18 Guard-Pak pre-column, Waters RCM 8 × 10 Module and Resolve reverse phase C18 column. The detector is an ESA Coulochem 5100A and model 5010 analytical cell that makes use of two porous graphite electrodes. Detector output was calibrated in arbitrary units of current and integrated on a Waters Model 745 data module. The mobile phase was delivered at 1 ml/min and consisted of potassium dihydrogenphosphate (21.8 g/l) adjusted with ortho-phosphoric acid to pH 3.0.

Chemical compounds in aqueous humor were identified by their retention time on the column and their unique signature on the analytical cell at a series of voltage settings as described in detail [14,5]. There is little sensitivity of the electrode system to the oxidized molecule cystine. Thus the values reported in acidic extracts subsequent to centrifugation can be considered that of free reduced molecule.

Discrimination of cysteine with a retention time of 3.1 min from hydrogen peroxide with a retention time of 2.9 min was problematic due to similar current-voltage curves for the two molecules. Ellman's reagent (DTNB: dithio-bis(2-nitrobenzoic acid) was employed with the final assay modified for small sample sizes [15]. DTNB complexed the sulfhydryl group of cysteine resulting in null voltage from the analytical cell for the 3.1 min peak.

Uric acid was identified as an HPLC peak at a retention time of 9.68 min that corresponded to a commercial standard with an identical current voltage curve. Uric acid was confirmed spectrophotometrically by exposure of the sample to uricase. Because uric acid absorbs @ 292 nm and the end products of the reaction (allantoin, CO_2 and H_2O_2) do not, decreased absorption is proportional to uric acid concentration. Alternatively, HPLC/EC was employed to confirm disappearance of uric acid after addition of uricase.

2.3. Acquisition, processing and transport of aqueous humor

Research animals were obtained and cared for in accordance with the recommendations of the OPRR Public Health Service Policy on Humane Care and Use of Laboratory Animals. At the DVA North Chicago Animal Research Facility, (12) SD outbred 4-month-old female rats, approximately 350 g, were euthanized, in a CO_2 chamber. Trans-corneal anterior-chamber paracentesis was performed with a 28-gauge needle/tuberculin syringe pre-filled with 50 μl HPLC mobile phase/N_2 gas. Aqueous humor (150 μl) was pooled from 12 rats ($n = 24$ eyes). Aqueous humor was also obtained from live mature white NZ rabbits (2.5–5 kg) using topical anesthesia and the same sampling procedure. Rabbits have a large anterior-chamber. Therefore, samples were harvested from single animals and combined with an equal volume of HPLC mobile phase, pH 3.

Human aqueous humor was recovered during extracapsular cataract-implant surgery from three elderly patients at Department of Veteran's Affairs Medical Center, N. Chicago, IL. Approximately 100 μl of pre-Healon® aqueous humor waste was placed in an eppendorf container pre-filled with 100 μl HPLC mobile phase.

Aqueous humor samples (animal or human) were covered with N_2 gas and frozen at -70°C.

2.4. Preparation of samples for HPLC/EC

All samples were processed within one week; this did not affect stability (data not shown). An HPLC/EC albumin standard (retention time = 5.5 min) was run, and no samples demonstrated a peak at this retention time, assuring that the blood-aqueous barrier was not compromised during paracentesis. Each sample was subsequently centrifuged 2 min at $14000 \times g$. For I/V curve construction, injections at multiple D_2 voltages

necessitated dilution of aqueous humor with HPLC mobile phase to a working volume of 400 μl. Electrochemical activity was assessed against Sigma standards prepared daily.

2.5. Synthetic aqueous humor

Synthetic aqueous humor incorporating a suitable buffer was formulated with double distilled water. The buffer system was selected to have minimal inherent confounding electrochemical activity. The synthetic aqueous humor contained two buffers: KH_2PO_4–H_3PO_4 and $NaHCO_3$–CO_2 gas. The electrolyte composition is based on published values (in mM) of 148 Na; K, 4; Cl, 134; HCO_3, 20; Ca, 2; AA, 1.0; pH 7.4 [16]. Concentrations of water soluble antioxidants determined in the first set of experiments for NZ White rabbit aqueous humor were used. In the 'hydrogen peroxide challenge' experiments, a bolus of hydrogen peroxide was added to this synthetic aqueous humor mixture of antioxidants and buffers to achieve a final concentration of 500 μM hydrogen peroxide. Challenge experiments subsequently were conducted with or without L-tyrosine in order to evaluate this amino acids role in a model human aqueous humor system.

2.6. Radiation apparatus

The design, construction and calibration of a unique apparatus for ultraviolet (UV B) irradiation of small volumes of biological fluid under temperature-controlled open atmospheric or closed cell conditions of defined Po_2 has been described [17]. Briefly, it consists of a custom designed UV transmissible quartz silica 3-port spectrophotometric cuvette coupled to a deuterium UV B source by UV transmissible fiber optics. The system stirs the fluid, regulates the temperature and monitors oxygen, pH, temperature and radiation. The apparatus produces a known photochemical reaction with a UV B photosensitizer and ascorbic acid.

Aliquots (400 μl) of freshly prepared synthetic aqueous humor or biologic samples, were irradiated under $P_{O_2} = 20.9\%$; $P_{CO_2} = 5\%$; 25°C in open and closed cuvette conditions. HPLC:EC was used to determine simultaneously the concentrations of aqueous humor antioxidants at 15 min intervals with/without UV B radiation, by withdrawing 25 μl samples from the cuvette periodically. A chelator (desferrioxamine B) was used initially, however consideration of the reaction thermodynamics revealed less confounding without a chelator. An aqueous humor endogenous UV B photosensitizer at a physiologic concentration (33 μM L-tryptophan) was included in select experiments.

2.7. Statistics

Repeated factors ANOVA significant P-values were determined where the repeated factor is 'time' and both a 'between sample' effect i.e. radiation/no radiation and 'within sample' effect i.e. the effect of time, were analyzed for significance using FASTAT (Evanston, IL) statistical software.

3. Results

Fig. 1 displays a representative chromatogram of aqueous humor from a mature NZ white rabbit depicting retention times and the relative peak height/area for each of five water soluble antioxidant species. A sixth peak represents the peptide cysteinyl–glycine, a breakdown product of glutathione. This appears on some chromatograms at a retention time of 4 min with an identical current voltage curve to a Sigma standard as previously described with vitreous humor [5].

Several lines of evidence suggest the 13 min chromatogram peak from each animal species is L-tyrosine. The peak is the same retention time to 0.01 min, and displays an identical current-voltage curve compared to the standard. There is also concordance with chemical characteristics. A Spectrum MWCO Centripor Concentrator® was used to arrive at a MW of less than 500 daltons. A pH titration curve (Fig. 2) indicates it contains one or more NH groups. Failed digestion with 10% trichloroacetic acid indicates it is not a protein. It

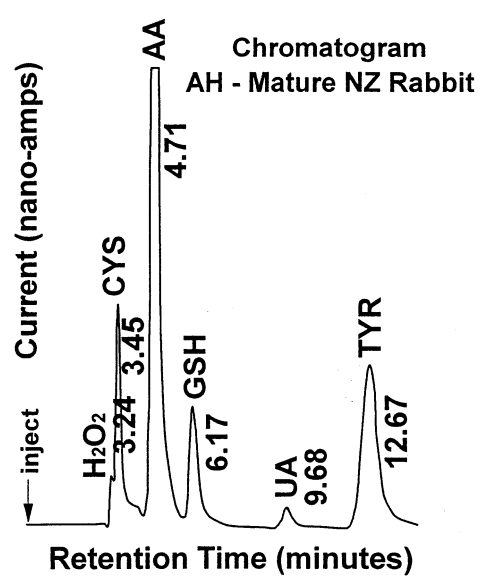

Fig. 1. Representative chromatogram of five species of low MW water soluble antioxidants in the aqueous humor of a mature NZ White Rabbit by HPLC/EC. Five major peaks elute in the following order: L-cysteine, L-ascorbic acid, glutathione, uric acid and L-tyrosine. A 6th peak: cysteinyl-glycine, a glutathione breakdown product, occasionally appears between ascorbic acid and glutathione.

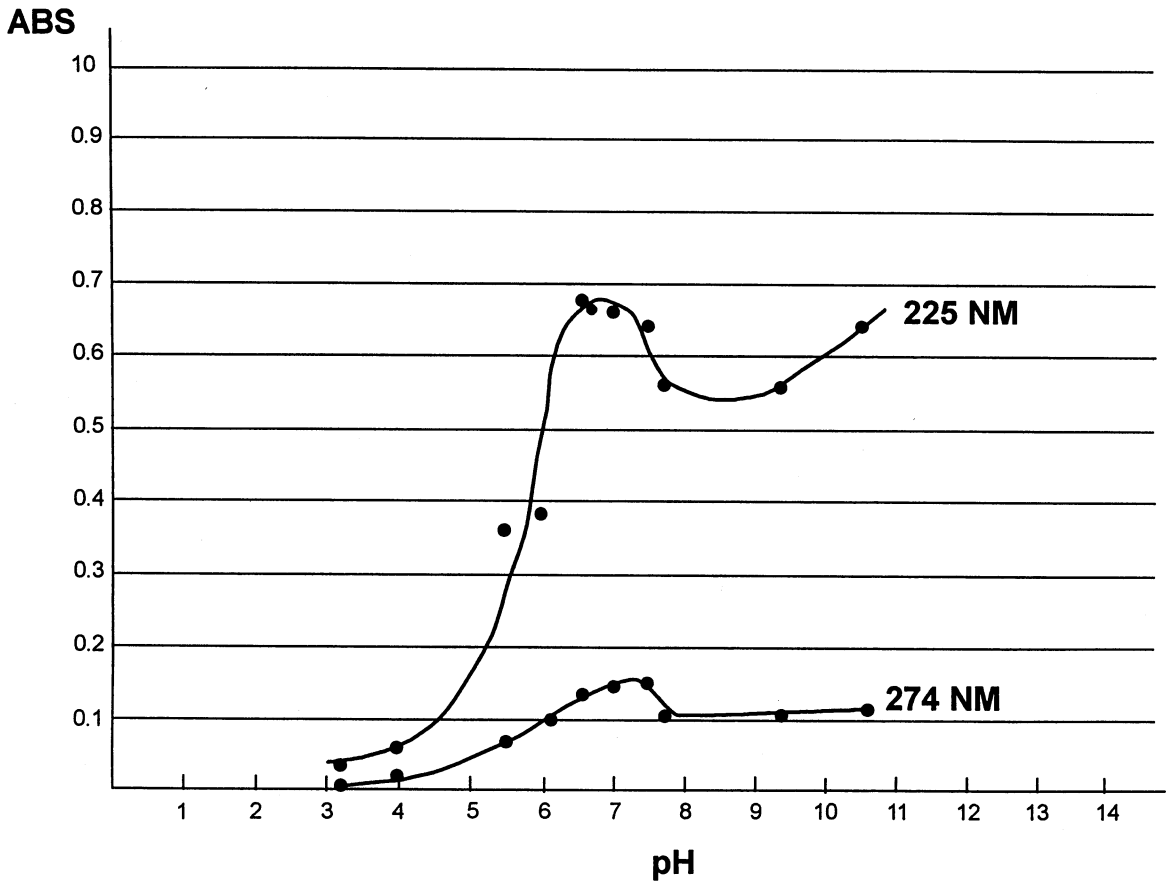

Fig. 2. The pH titration curve of the aqueous humor 13 min retention time peak L-tyrosine; mature NZ White rabbit sample.

is not a sulfhydryl since it does not complex with DTNB as does L-cysteine.

Chromatograms of candidate electrochemically active aqueous humor molecules revealed no matching current-voltage signatures except for L-tyrosine. Aqueous humor molecules evaluated included proteins (i.e. transferrin), sugars, metabolic byproducts (i.e. pyruvate), bipeptides, purine metabolites and reduced nucleotides [18]. Also, the 13 min peak from NZ mature rabbit aqueous humor is eliminated with mushroom tyrosinase (Fig. 3). The 13 min fraction was also shown in preliminary studies to be of low MW with direct chemical ionization (DCI) and fast atomic bombardment (FAB) using a Finnigan TSQ70B triple stage quadrupole mass spectrometer.

The OH group on L-tyrosine is para. It is not the structural isomer M-tyrosine which has a retention time of 16.5 min (not shown).

3.1. Comparative physiology

Table 1 displays average concentrations of water soluble antioxidants for each mammal. The concentration of free reduced cysteine in rabbit and human aqueous humor is similar to that reported in the plasma of healthy humans (9 μM) [19].

In agreement with Reiss, in both diurnal rabbits and humans, ascorbic acid is the major water soluble antioxidant and the nocturnal rat has a low concentration of aqueous humor ascorbic acid [20]. However, in the nocturnal rat, our data demonstrate a significantly greater concentration of glutathione compared to values for rabbits and human patients. Low aqueous humor glutathione concentration in diurnal species agree with values of 16 μM for rabbits and 3.5 μM for cataract patients [21]. Such low aqueous humor glutathione concentration from diurnal species also contrast to high millimolar cytoplasmic values. Furthermore glutathione concentration reported herein and by Chakrapani for cataract patients, is a few times higher than that reported in 'normal' human aqueous humor (1.9 μM) [22].

Our value for uric acid concentration in humans is similar to that reported for cataract patients (47–101 μM) by two groups [23,24]. The significance of higher aqueous humor uric acid concentrations (in humans) parallels that found by Ames et al. in plasma. They proposed that a major factor in lengthening human life span and decreasing age related cancer rates is plasma uric acid level, which increased markedly during primate evolution [25]. Rat uric acid concentration is close to that reported for Wistar rats (3 μM) [26] and lower than in rabbits.

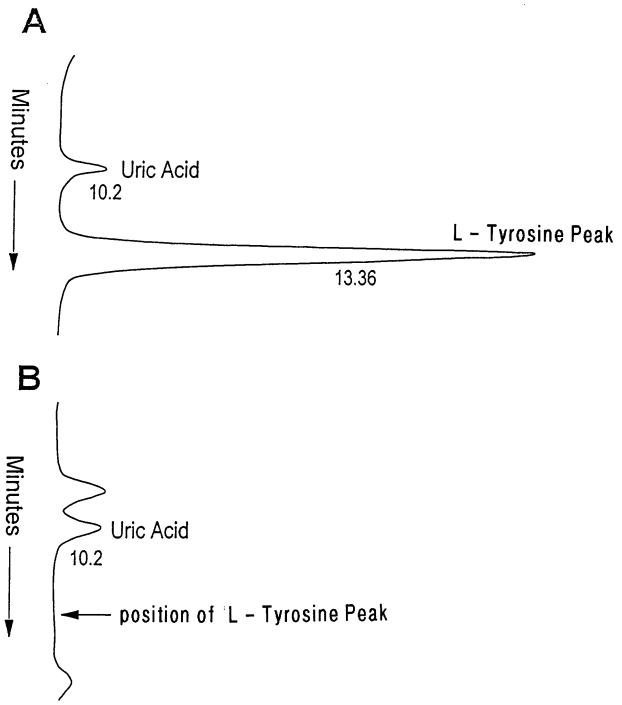

Fig. 3. Chromatogram demonstrating elimination of L-tyrosine with the enzyme mushroom tyrosinase. This is a copper containing oxidase enzyme widely distributed in plants, animals and man having activity for catechols and cresol. (A) mature NZ White rabbit aqueous humor (B) same, but with mushroom tyrosinase added.

3.1.1. Radiation experiments

Single component (cysteine, ascorbic acid, glutathione, uric acid or tyrosine) and multicomponent experiments with and without a chelator (0.1 mM desferal) or an endogenous UV B photosensitizer (33 μM L-tryptophan) elicited no change in concentration from control (no radiation) when a UV B dose of 30 mJ/cm^2 per h of radiation was applied. This is the equivalent to the dose of UV B found in 5.5 h of sunlight [17]. In pooled human aqueous humor, there was also no change in the concentration of any electrochemically active species upon irradiation of the cuvette. These results are consistent with the finding of no change in oxygen concentration or hydrogen peroxide when ascorbic acid was irradiated with/without a UV B photosensitizer. For example if oxygen is reduced to water as ascorbic acid is oxidized to dehydroascorbic acid (probably going through H_2O_2), then 2 moles of ascorbic acid are consumed with 1 mole of oxygen.

3.2. H_2O_2 challenge or 'Hierarchy' experiments

The rationale for these experiments was to use an alternative oxidant stress, hydrogen peroxide, which is an end product of metabolic and photochemical reactions. These experiments were undertaken since a physiologic dose of UV B radiation did not deplete each antioxidant, lower P_{O_2}, nor generate a significant concentration of hydrogen peroxide. The upper limit of safety within the eye is unknown, but high concentrations of hydrogen peroxide lead to ciliary process swelling, increased iris blood vessel permeability and lens damage. Hydrogen peroxide at 200 μM is considered the upper limit of safety for the corneal endothelium; concentrations as high as 500 μM have been found in the aqueous humor of some cataract patients. A 500 μM hydrogen peroxide 'challenge' was selected. Synthetic human aqueous humor and hydrogen peroxide are quite stable for at least 1 h at room temperature (not shown).

A bolus of hydrogen peroxide was added at $t = 0$ min to either synthetic aqueous humor or synthetic aqueous humor without L-tyrosine, and the concentrations of low MW water soluble antioxidants and hydrogen peroxide were followed for 1 h. The hierarchy of human aqueous humor water soluble antioxidants to a hydrogen peroxide challenge (without radiation and high oxygen) was: CYS > GSH > AA > UA > TYR, ($n = 3$ trials). This result agrees quite well with Buettner's predicted 'pecking order' or hierarchy, based on thermodynamics [12]. Hydrogen peroxide being a strong oxidant with a positive one electron redox potential oxidized the thiols quickly because of a combination of auto-oxidation, a redox potential more negative than hydrogen peroxide and their low concentration. Ascorbic acid being a strong reductant with a low positive redox potential was next to be oxidized. Uric acid and tyrosine were last.

Fig. 4 illustrates that ascorbic acid tends to oxidize more rapidly in the presence of L-tyrosine ($P = 0.10$;

Table 1
Concentration (μM) of water soluble aqueous humor antioxidants in mammals: mature New Zealand White rabbits; young Sprague–Dawley rats; elderly human cataract patients

	Mammalian Aqueous Humor (μM)				
	CYS	AA	GSH	UA	TYR
New Zealand Rabbits ($n = 3$)	15.7 ± 4.7	1541 ± 333	25.3 ± 3.8	32.2 ± 9.4	101 [14]
Sprague–Dawley Rats ($n = 24$) pooled	63.0	17.9	125	11.2	nd
Human cataract patients ($n = 3$) pooled	14.3	530	5.5	43	78

Values calculated against Sigma standards as described in the text and reference [5,39].
Values expressed as mean ± S.D. nd, not determined, as identity of L-tyrosine peak not established.

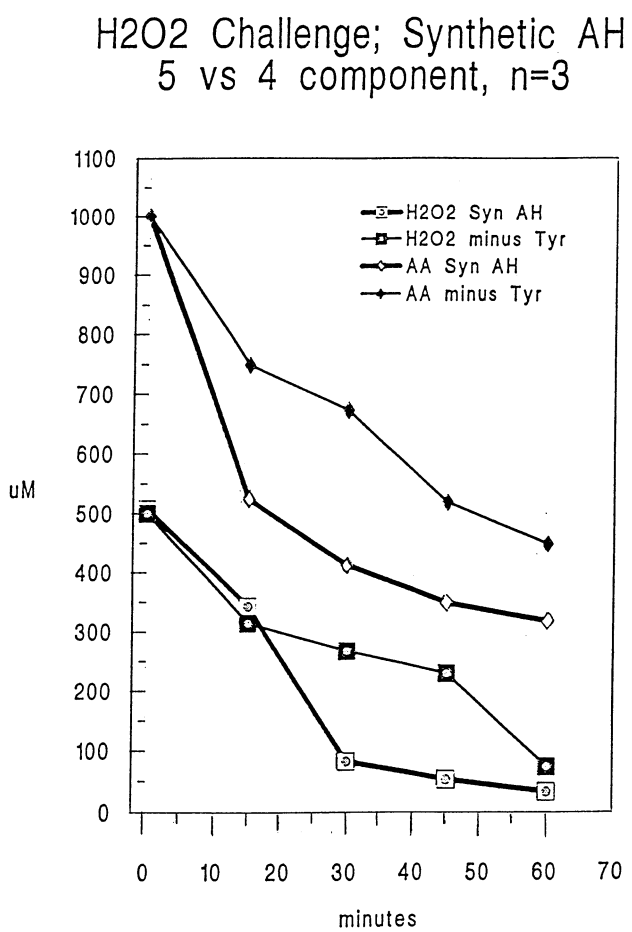

Fig. 4. Time course of ascorbic acid and H_2O_2 decay following addition of a bolus of H_2O_2 to synthetic human aqueous humor with/without L-tyrosine. Ascorbic acid oxidation is both faster and greater in the synthetic aqueous humor mixture ($P = 0.10$; repeated factors ANOVA).

repeated factors ANOVA). This finding is in agreement with empirical observations and one electron reduction thermodynamic predictions. That is, the redox pair tyrosinol-O$^{\bullet}$, H^+/tyrosinol-OH has an $E_0 = +0.80$ V while AA/H^+/AA monoanion (vitamin C) has a redox potential $E_0 = +0.32$ v.

4. Discussion

Advantages of HPLC/EC are its high sensitivity, accuracy, convenience, linearity and the ability to determine concentrations of several electrochemically active molecules in a single assay. Five major water soluble antioxidants have been identified and their concentrations in three species of mammalian aqueous humor determined. We have previously identified these same molecules in rabbit, cow and rat vitreous humor [5] and in human tears collected at low and high flow rates [6]. All five molecules are conserved by the kidney.

The first report of the aqueous humor amino acid cysteine in mammals (monkey) [27] has been confirmed. Cysteine is the rate limiting precursor for glutathione synthesis, and one breakdown product. Cysteine and derivatives were also the first radioprotectors discovered [28]. Both cellular thiol antioxidants, cysteine and glutathione, are more concentrated in the nocturnal rat than in two diurnal species (rabbits and humans) which maintain a high concentration of ascorbic acid.

Uric acid is present in the aqueous humor of each type of mammal. Two review articles [25,29] suggest a significant role for uric acid in primate plasma, possibly in regulating the redox state of the glutathione-ascorbic acid system [30,31]. However, human aqueous humor uric acid is similar in concentration to that of cerebrospinal fluid and only a fraction of human plasma concentration, signifying that uric acid does not easily enter the anterior-chamber. This contrasts with ascorbic acid which is actively transported from the ciliary epithelium.

The electrochemically active amino acid tyrosine is of second highest concentration compared to other aqueous humor water soluble antioxidants in humans. It's properties have been evaluated in synthetic aqueous humor, where it tends to act as an oxidant in agreement with empirical observations and one electron reduction potential-thermodynamic predictions. Tyrosine has recently been mentioned as a biological antioxidant [32].

Tyrosine is a hydroxyl radical scavenger, singlet oxygen quencher and weak photosensitizer [13]. These properties are distinct from it's well known roles as an enzyme co-factor in DNA synthesis, an amino acid in signal transduction and a precursor for melanin and catecholamines. In that the aqueous humor/plasma ratio of tyrosine is 1.74 in rabbits; 1.26 in monkeys and 1.84 in human [16], tyrosine appears not to undergo active transport from the ciliary epithelium.

The physiologic equivalent of 5.5 h of UV B aqueous humor sunlight hours and the in vitro equivalent of 100% oxygenation did not result in deterioration of each water soluble antioxidant component singly, or when combined in synthetic or biologic aqueous humor. Addition of the endogenous UV B photosensitizer L-tryptophan at it's physiologic aqueous humor concentration did not result in deterioration of synthetic aqueous humor water soluble antioxidant components despite the fact that L-tryptophan is photochemically altered [33,17]. Further, hydrogen peroxide was not produced to a significant degree by the oxidation of ascorbic acid when a chelator was present.

If the high concentration of hydrogen peroxide reported in some cataract patients [34] is derived from a UV B mediated photochemical reaction, the process is not initiated in our system. The presence of other photosensitizers in vivo may be significant.

Examination of UV B damage thresholds to other relevant contiguous tissues i.e. cornea [35] and lens [36] reveals that physiologic relevant doses of UV B radiation in the order of 30–60 mJ/cm^2 would primarily impact DNA. Indeed, 14 major studies link UV B exposure and cataract [37]. Further teleological arguments based upon (1) a lens gamma glutamyl cycle that is sluggish, (2) specific lens epithelial cell transporters for thiols, and (3) high aqueous humor turnover rate, suggest that it would be disadvantageous for radiation to destroy thiols. This is the result we found in our radiation experiments. That is, a fortunate consequence of aqueous humor thiols not being damaged by UV B solar radiation is that they remain available to the lens, particularly the anterior lens epithelium. Glutathione is initially depleted in every known model of cataract. In addition to maintaining a reduced environment in the anterior-chamber, i.e. for protection of glutathione, a recent publication suggests that the role of ascorbic acid at high concentration in the aqueous humor of diurnal animals is to quench tryptophan-induced fluorescence [38].

Water soluble antioxidant 'hierarchy experiments' with high oxygen partial pressure and without radiation demonstrate that 90% of a bolus of 500 μM hydrogen peroxide is eliminated within 1 h upon addition to synthetic aqueous humor (lacking catalase or peroxidase enzymes). The hierarchy of human aqueous humor water soluble antioxidants to a hydrogen peroxide challenge (without radiation and high oxygen) is: CYS > GSH > AA > UA > TYR.

In conclusion, mammalian aqueous humor, like vitreous humor, contains multiple low MW water soluble electrochemically active components. This complexity must be recognized in choosing appropriate animal models to evaluate aqueous humor antioxidant defense.

Acknowledgements

Thanks to Moris Eisen,PhD Technion University Department of Biochemistry, Haifa, Israel for mass spectroscopy. We acknowledge NIH grant EY07320, Illinois Society for the Prevention of Blindness and the American Optometric Foundation.

References

[1] Rose R. Transport of ascorbic acid and other water-soluble vitamins. Biochim Biophys Acta 1987;947:335–66.
[2] Rose R. Renal metabolism of the oxidized form of ascorbic acid (Dehydro-L-ascorbic acid). Am J Physiol 1989;256:f52–6.
[3] Rose R, Bode A. Tissue-mediated regeneration of ascorbic acid: Is the process enzymatic? Enzyme 1992;46:196–203.
[4] Rose R, Bode AM. Biology of free radical scavengers: an evaluation of ascorbate. FASEB J 1993;7:1135–42.
[5] Rose R, Gogia R, Richer S. Properties of electrochemically active components in mammalian vitreous humor. Exp Eye Res 1997;64:807–12.
[6] Gogia R, Rose R, Richer S. Tear fluid content of electrochemically active components including antioxidants. Curr Eye Res 1998;17:257–63.
[7] Rose R. Ascorbic acid metabolism in protection against free radicals: A radiation model. Biochem Biophys Res Comm 1990;169(2):430–6.
[8] Rose R, Bode A. Mini Review,Ocular ascorbate transport and metabolism. Comp Biochem Physiol A 1991;100:273–85.
[9] Taylor A, Jacques PF, Epstein EM. Relations among aging, antioxidant status and cataract. Am J Clin Nutr 1995;62(suppl):1439–47.
[10] Spector A, Wang G, Wang R, Garner WH, Moll H. The prevention of cataract caused by oxidative stress in cultured rat lenses to hydrogen peroxide and photochemically induced cataract. Curr Eye Res 1993;12(2):163–79.
[11] Spector A, Wang GM, Wang RR. Photochemically induced cataracts in rat lenses can be prevented by AL-3823A, a glutathione peroxidase mimic. Proc Natl Acad Sci USA 1993;90(16):7485–9.
[12] Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch Biochem Biophys 1993;300(2):535–43.
[13] Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. second ed. Oxford: Oxford University Press, 1991.
[14] Rose R, Bode A. Analysis of water soluble antioxidants by high pressure liquid chromatography. Biochem J 1995;306:101–5.
[15] Deakin H. A sensitive sulfhydral agent. Biochem J 1963;89:296.
[16] Hart Jr, WmM, editor. Adler's Physiology of the Eye, St. Louis, MO: Mosby Year Book, 1992.
[17] Richer S, Rose R, Gogia R. An apparatus for UV B irradiation of small volumes of biological fluid under controlled oxygen tension. Optom Vis Sci 1996;73:683–8.
[18] Richer S. Mammalian Aqueous Humor Water Soluble Antioxidants. PhD, FUHS-Chicago Medical School/University Microfiche; Ann Arbor, MI; # 97–04848, 1996.
[19] Mansoor M, Svardal A, Ueland P. Determination of the in vivo redox status of cysteine, cysteinylglycine, homocysteine, and glutathione in human plasma. Anal Biochem 1992;200:218–29.
[20] Reiss GR. Ascorbic acid levels in the aqueous humor of nocturnal and diurnal mammals. Arch Ophthalmol 1986;104:753–5.
[21] Chakrapani B, Yedavally S, Leverenz V, Giblin F, Reddy V. Simultaneous measurement of reduced and oxidized glutathione in human aqueous humor and cataracts by electrochemical detection. Ophthalmic Res 1995;27(suppl 1):69–77.
[22] Riley M, Meyer R, Yates E. Glutathione in the aqueous humor of human and other species. Invest Ophthalmol Vis Sci 1980;19:94–6.
[23] Lam K, Liu K, Yee R, Lee P. Detection of uric acid in aqueous humor by high pressure liquid chromatography. Curr Eye Res 1983;2:645–9.
[24] Mendelsohn M, Abramson D, Notis C, Senft S, Servodidio P, Gamache P. Retinoblastoma: uric acid levels in aqueous humor (ARVO poster # 2263). Ophthalmic Oncology Center, New York Hospital-Cornell Medical Center, Hawaii Eye Center, King Khaled Eye Hospital, Saudia Arabia and ESA laboratories, MA, 1995.
[25] Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc Natl Acad Sci 1981;78(11):6858–62.
[26] Bonney CH, Lam KW, Fong D. Integrity of the blood-aqueous barrier in hyperuricemia. Ann Ophthalmol 1988;20:140–2.
[27] Gaasterland D. Rhesus monkey aqueous humor composition and a primate ocular perfusate. Invest Ophthalmol Vis Sci 1979;18(11):1139–50.

[28] Hall EJ. Radiobiology for The Radiologist. 4th ed. Philadelphia: JB Lippincott, 1994.
[29] Becker BF, Reinholz N, Leipert B, Raschke P, Permanetter B, Gerlach E. Role of uric acid as an endogenous radical scavenger and antioxidant. Chest 1991;100(3):176S–9S.
[30] Sevanian A, Davies K, Hochstein P. Serum urate as an antioxidant for ascorbic acid. Am J Clin Nutr 1991;54:1129S–134.
[31] Meister A. Glutathione-Ascorbic acid antioxidant System in animals—Minireview. Biol Chem 1994;269(13):9397–400.
[32] Whiteman M, Halliwell B. Protection against peroxynitrate-dependent tyrosine nitration and alpha 1-antiproteinase inactivation by ascorbic acid. A comparison with other biological antioxidants. Free Radic Res 1996;25:275–83.
[33] Hightower K, McCready J. Mechanisms involved in cataract development following near-ultraviolet radiation of cultured lenses. Curr Eye Res 1992;11(7):679–89.
[34] Spector A, Garner WH. Hydrogen peroxide and human cataract. Exp Eye Res 1981;33:673–81.
[35] Ringvold A. Changes of corneal endothelium after UV radiation. Acta Ophthalmol Cph 1982;60:41–63.
[36] Andley U, Weber J. Ultraviolet action spectra for photobiological effects in cultured human epithelial cells. Photochem Photobiol 1995;62(5):840–6.
[37] McCarty C, Taylor H. Recent developments in vision research: Light damage in cataract. Invest Ophthalmol Vis Sci 1996;37(9):1720–3.
[38] Ringvold A. The significance of ascorbate in the aqueous humor protection against UV-A and UV-B. Exp Eye Res 1996;62:261–4.
[39] Ehlers N, Schonheyder F. Concentration of amino acids in aqueous humor and plasma in congenital cataracts. Acta Ophthalmol 1974;123S:179–89.